A COMPARISON OF THE RATE OF BEAT IN PORTIONS OF THE HEART FROM 4 AND 6 DAY OLD CHICK EMBRYOS GROWN IN VITRO

A THESIS

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CHAPTER I

INTRODUCTION

In vitro observations have been very important in the study of the embryonic heart. This investigation was performed in order to make a comparison of the rate of beat in explanted portions of the heart from 4 and 6 day old chick embryos. The heart was dissected from the embryo and divided into three portions.

Explants from portions of the heart of 4 and 6 day old chick embryos showed different rates of contraction. The sinus venosus and the atrium exhibited a higher rate of contraction than did the ventricle. The rate of beat of the atrium was slightly higher than that of the sinus venosus. Each explant exhibited contraction from the first to the 5th day. In many cases there was a decrease in the rate of beat each day following the first day.

CHAPTER II

REVIEW OF LITERATURE

Many advances have been made on the study of heart activity through tissue culture methods. This method allows for continuous observations of the explanted portions of the embryonic heart. In this manner the first appearance of contraction in the early embryonic heart of the chick may be observed. According to Patten ('49) Haller; Schenk; Wernicke; and Fano and Badano, were among the earliest investigators who worked on the actual beginning of the heart beat *in vitro* and *in ovo*. Haller saw the heart beating and the blood in chick embryos 45 hrs. old. Schenk excised the heart of a three day chick embryo and observed the heart beat outside the body. The rate of heart beat increased after the temperature was raised. The heart was cut into fragments and after a short period each fragment resumed contraction. Wernicke observed that the rate of heart beat increased during the early stages of development and attempted to determine the effect of various chemical solutions on the rate of pulsation of the heart. Fano and Badano crushed the heart of the chick embryo at the atrium and ventricular connection and noted that the atrium continued to beat, while the ventricle stopped beating. When the beating was resumed in the ventricle the rate was much slower than in the atrium.

Sabin ('20) observed the time at which the first heart beat occurred *in vitro*. It was noted that the first heart beat occurred in the ventricular portion and not in the atrium. In hanging drop cultures, the first beats were observed at the 10 somite stage in the same place. The first indication of activity was a simple movement along the right margin of the ventricle. The beat started slowly with the entire wall of the ventricle moving.

According to Patten ('49), Olivo obtained results similar to those of Sabin's. It was reported that the first contractions occurred in small sections of the myocardium of 9 somite embryos. The first contractions were greater on the left side than on the right side.

Lewis ('24) studied heart muscle tissue in vitro from chick embryos 28 to 30 hrs. old. It was found that the tissue would contract continuously for an hour or longer. In several cases only portions of the tissues were observed to contract, and in some cases the entire piece of tissue showed contraction. Some contraction was observed in most of the material studied. The heart muscle tissue contracted very rapidly. These observations were not confirmed by Cohn ('25) who observed the embryonic heart in vitro and in ovo. Observations were made on hearts of embryos from two days and 18 hrs. old. The heart was divided for explantation into the atrium, ventricle and bulbus arterious. Changes were observed in the rate of contraction during the period of incubation. There were certain factors believed to be responsible for these changes. According to him these factors included: changes in the general metabolism of the body; in the structure of the heart muscle, and in the activity of the muscle tissue. Changes in temperature were observed to influence the rate of contraction of the heart. The rate at which the heart beat was consistent at each stage of development. Cohn attempted to find the site in the heart which controlled the rate of its' contraction. It was concluded that areas throughout the cardiac tube and a small area in the dorsal wall of the right auricle controlled the rate.

According to Hyman ('27) observations made on the heart of the 9 to 10 somite stage embryo in ovo showed that the right border of it was sensitive to toxic substances. She suggested that this indicated a higher metabolic rate on the right side of the heart at this stage. She postulated that the

right border of the heart may be active in cellular proliferation. This suggested to her that the part of the heart having the highest metabolic rate is the part of the heart showing the first signs of contraction.

Johnstone ('25) continued the work of Sabin ('20). Observations were made in vitro on the hearts of chick embryos at the 9 somite stage. It was found that the first contractions of the heart occurred on the right border of the primitive ventricle. From this point the contraction was observed moving out into a larger area of the ventricle. The movement was in the direction of the arterial end rather than the venous end. Eventually the entire primitive ventricle contracted. At this time no signs of contraction were observed in the sinus venosus. Johnstone attempted to determine whether or not the sinus venosus initiated the stimulus for the beat in the ventricle, since no beat was located in it. An attempt was made to show that the ventricular beat was independent of any type of stimulus from the sinus venosus. The sinus venosus was tied off from the ventricle. This excluded the sinus venosus as the pace-making center for the ventricle of the early embryonic heart. Observations indicated that the sinus venosus did not initiate the contractions of the heart until the end of the third or beginning of the 4th day, when it started to beat. He tied off a portion of the heart at the junction of the sinus venosus and the atrium. A heart block was produced, indicating according to him, that by the 4th day of incubation the pace-making center was located in the sinus venosus.

Patten and Kramer ('33) found that the sinus venosus was the last part of the heart to form. This was believed to be an important factor in locating the origin of the first heart beats. This concept was put forth by Sabin ('20) and Johnstone ('25). They suggested that the first heart beat occurred in the ventricle and not in the sinus venosus. Patten and Kramer used

hanging drop cultures and observed the first heart beat *in vitro*, at the 9 to 10 somite stage. The embryo was removed from the egg and mounted on the cover-glass with strips of filter paper. Large chambers made of plate-glass strips were substituted for depression slides. Humidity was maintained in the chamber by placing rolls of water soaked filter paper in the corners. The embryos in the large chambers lived longer than those placed on depression slides. The first contractions were observed in the ventricle and not in the atrium. The rate of contraction in the ventricle was very slow. Following the initiation of contraction in the ventricle, the atrium contracted, the rate of which was higher than that of the ventricle. The rate of contraction of the total heart was increased as a result of this higher rate of contraction in the atrium. When the blood started to circulate in the embryonic heart the sinus venosus started contracting at a higher rate than either the atrium or the ventricle. This further increased the total rate of contraction of the heart.

Several factors were observed at the 9 to 10 somite stage, in connection with the first heart beats. Lewis ('19) observed the first appearance of cross striations with the first contractions of heart muscle. Allen ('19) observed the first appearance of glycogen in the heart muscles. It was suggested that the appearance of glycogen at this stage in the developing heart muscle may be connected with the first contractions of the heart. According to Allen, this is believed to be true, in that no glycogen was observed in the developing heart muscle until the initiation of contraction.

Goss ('37) studied the initiation of contraction in the hearts of rat embryos *in vitro*. The embryos were $9\frac{1}{2}$ days old. Contractions were observed at the junction of the atrium and ventricle. There was neither circulation of blood through the atrium, nor were there any noticeable signs of contraction.

Goss ('40) studied the cytological structures which appear at the time of the initiation of the heart beat in rat embryos. Heart muscle was observed before and after the first contractions. Embryos were fixed in Zenker's solution at the beginning of the first contractions and after circulation was well established. Specialized cytological structures which included fibrillae and cross striations were observed after contraction of the heart was well established. Similar observations were made by Copenhaver ('39) who studied amblystoma embryos stage 33, in vivo. The embryos were placed in Ringer's solution and pieces of ectoderm and mesoderm were cut away in order to allow the heart to be studied by direct observation. The earliest contractions were observed in the anterior portion of the heart, which develops into the ventricle and bulbus arteriosus.

CHAPTER III

MATERIALS AND METHODS

The single cover-glass method was used in these experiments; the depression slides and cover-glasses were of uniform size. The eggs used were from White Rock hens secured from the Georgia State Hatchery, Atlanta, Georgia. The eggs were dated with a pencil and placed in an incubator at a temperature of 37°C. for 4 and 6 days. The eggs were turned each day to prevent the embryo from adhering to the shell membrane. Humidity was maintained in the incubator by placing tap water in a metal container which contained a cloth to absorb the water. The media used in the experiments included: Earle's physiological salt solution, chick embryonic extract and chicken plasma. The Earle's solution and dessicated plasma were obtained from the Difco Laboratories, Inc., whereas the embryonic extract was prepared in the laboratory from 9 - 12 day old chick embryos.

All of the glassware and instruments used in the experiments were sterilized before each explantation. The instruments and glassware were packed in the order that they were used, in metal cannisters. They were then sterilized by dry heat for two hours, at a temperature of 140 - 150°C.

The embryos were removed from the eggs and placed in a Petri dish which contained Earle's solution. The heart was removed from the embryo with iridectomy scissors under a sterioscopic microscope, and placed in a Maximow slide, the well of which was filled with Earle's solution. The Maximow slide containing the heart was placed in a Petri dish. The heart was divided into three portions: the atrium, ventricle, and sinus venosus. Each of these were separated and divided into smaller fragments. Five cover-glasses were placed in each of three Petri dishes with watchmaker forceps. Equal drops of plasma

and extract were placed on each cover-glass with a glass pipette. The drops were mixed on the cover-glasses with a scalpel and a fragment of tissue was placed immediately in the medium. After coagulation each explant was inverted over the depression slide, sealed with a mixture of paraffin and vaseline and incubated at 37°C.

The explants were studied individually. The preparation was placed in a stage incubator under the microscope. Observations were made each day for 5 days after explantation. The rates of beat of each explant (i.e., the atrium, ventricle, and sinus venosus) were observed and recorded throughout the five day period. A stop watch and a standard counter were used to count the number of beats per minute.

CHAPTER IV

EXPERIMENTAL RESULTS

The total number of explants from each portion of the heart of 4 day old chick embryos were as follows. There were 52 explants of the sinus venosus, 49 of the ventricle, and 46 of the atrium. The total number of explants from 6 day old chick embryos were as follows. There were 48 explants of the sinus venosus, 46 of the ventricle and 43 of the atrium. Explants from portions of the heart of 4 and 6 day old chick embryos exhibited different rates of contraction. This contraction was observed to persist from the first to the 5th day under the conditions of this experiment. Various explants were observed to beat intermittently.

In the explants from the 4 day old chick embryos the rate of beat of the atrium, ventricle and sinus venosus was observed to be different. The atrium and sinus venosus exhibited a high rate of beat on the first and second day than on the following days. In the case of the atrium there was a slight increase in the rate the second day with a decrease each successive day. The sinus venosus showed a decrease each successive day up to the 4th day, and on the 5th day there was an increase. The ventricle displayed a lower rate of beat than did the atrium or sinus venosus. There was a decrease in the rate each day following the first day (Table 1).

Corresponding explants from the 6 day old embryos showed a rate of beat that was lower than that of the 4 day old embryos. Explants of the atrium and sinus venosus displayed a higher rate of beat on the first and second day, after which there was a decrease each consecutive day. The ventricle showed a lower rate of beat than did the atrium and sinus venosus (Table 1).

In explants of the sinus venosus and atrium from 4 day old chick embryos the average number of beats per minute were higher than that of the ventricle. The average rate of beat of the atrium was slightly higher than that of the sinus venosus. In each case there was a decrease each successive day. In explants of the sinus venosus and atrium from 6 day old chick embryos the rate of beat was higher than that of the ventricle. The average rate of the atrium was slightly higher than that of the sinus venosus. Each portion showed a decrease in the average rate each consecutive day (Table 2).

CHAPTER V

DISCUSSION

In many cases the explants of various portions of the heart were not beating from the beginning of observations to the end of the 5th day. The observations in these experiments indicated that the rates of beat in 4 and 6 day old explants of the sinus venosus and atrium, were higher than those of the ventricle. Several investigators have observed similar results. Burrows ('12) studied the rate of beat in portions of the heart from chick embryos. He found that the rate of beat in the atrium was greater than that of the ventricle. Cohn ('25) and Johnstone ('25) made *in vitro* studies of portions of the heart of chick embryos and observed a higher rate of contraction in the sinus venosus and atrium than in the ventricle. They suggested that this higher rate was due to the gradient in the rates of contraction from the cephalic portion of the heart to the caudal end. This gradient was believed to be present within each chamber of the heart. Barry ('42) made *in ovo* studies on the heart of chicken embryos. He suggested that there was a gradient of inherent rate of contraction between each chamber of the heart and called it intercameral. A gradient was described within each chamber which was called intracameral. Each portion had a higher rate than the ones cephalic to it, and lower than those caudal to it. This may account for the beating of only a portion of the explants in this investigation. Patten and Kramer ('33) made *in vitro* studies of the heart from chick embryos. They found that there was a change in the inherent rate of contraction of various portions of the heart. The difference in the rate of contraction of various portions of the explanted heart tissue may be explained on the basis

of the shifts in the pace-maker. Paff ('35) explanted embryonic chick hearts, and found that the rate of beat in the sino-atrial portion of the heart was higher than that of the ventricle.

Szepsenswol ('46) presented some interesting results which may provide an explanation for the results of this investigation. The activities of heart and skeletal muscle from chick embryos were studied in vitro. He suggested that heart tissue possesses from the beginning, a tendency to organize itself into a single functional unit in which the rate of contraction is determined by that portion of the heart with the highest frequency. Differentiation was observed in the portion of the heart with the highest frequency, during the early stages of embryonic development. There was a reduction in the frequency of heart beat of each portion of the heart. This reduction started at that portion of the heart with the highest frequency of beat and eventually shifted to that portion of the heart with the lowest frequency. He described the frequency of contraction as being gradually shifted from the atrium to the distal portion of the ventricle. The manner in which the explants functioned was believed to be due to the action of the muscle fibers of the heart. The muscle fibers of the heart with the higher frequencies have dominant rates of beat over those with the lower frequencies.

Goss ('38) made in vitro studies of portions of the heart from rat embryos and obtained results similar to those of this investigation. The rate of beat in the sinus venosus and atrium was greater than that of the ventricle. Copenhaver ('39) studied the hearts of amblystoma embryos in vivo and obtained similar results. Both investigators suggested that the actual rate of beat of each chamber was present at successive stages of development. This indicated that the inherent rate at any level other than the pace-maker decreased during the older embryonic stages.

In most cases in this investigation there was a decrease in the rate of beat each day. Copenhaver ('39) and Cohn ('25) made *in vitro* studies of chick embryos. They obtained similar results in their experiments. According to them the muscles of the various portions of the heart tend to lose their spontaneous beat with age. This was believed to be due to degenerative changes produced in the myocardium. In many cases as presented here the contraction of various portions of the heart ceased although the explant was still living, and showed extensive growth of the myocardium. Barry ('42) suggested that the inherent rate of contraction in a single portion of the heart may decrease with age.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Portions of the heart (i.e., atrium, ventricle, and sinus venosus) of 4 and 6 day old embryos were grown *in vitro*. Explants were observed from one to 5 days. Several explants exhibited growth without contraction.

2. The rate of beat in each portion of the heart was observed to decrease each day in the majority of the explants.

3. The rates and ranges of beat of the various portions of the heart were observed to be different. In most cases the rate of beat of explants of the atrium was only slightly higher than the rate of beat of the sinus venosus. The explants of the atrium and sinus venosus had a higher rate of contraction than did the ventricle.

4. These observations are believed to be a result of several factors: morphological differentiation; inherent rates of contraction; gradients in the rates of contraction, and differences in the frequency of contraction in various portions of the heart.

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TABLE 1

RANGE OF BEAT OF EACH PORTION OF THE HEART

4 DAY OLD CHICK EMBRYOS

	1st day Beats per/min.	2nd day Beats per/min.	3rd day Beats per/min.	4th day Beats per/min.	5th day Beats per/min.
Atrium	7 - 140	0 - 144	0 - 63	0 - 33	0 - 13
Ventricle	6 - 78	0 - 52	0 - 48	0 - 0	0 - 0
Sinus Venosus	12 - 135	9 - 131	0 - 71	0 - 62	0 - 71

6 DAY OLD CHICK EMBRYOS

	1st day Beats per/min.	2nd day Beats per/min.	3rd day Beats per/min.	4th day Beats per/min.	5th day Beats per/min.
Atrium	0 - 115	0 - 134	0 - 78	0 - 0	0 - 0
Ventricle	0 - 72	0 - 49	0 - 34	0 - 0	0 - 0
Sinus Venosus	4 - 107	0 - 122	0 - 45	0 - 4	0 - 0

TABLE 2

AVERAGE NUMBER OF BEATS PER MINUTE

4 DAY OLD CHICK EMBRYOS

	1st day Av. Rate of beats per/min.	2nd day Av. Rate of beats per/min.	3rd day Av. Rate of beats per/min.	4th day Av. Rate of beats per/min.	5th day Av. Rate of beats per/min.
Sinus Venosus	64.3	54.9	18.0	5.3	2.8
Ventricle	50.2	34.1	7.0	0	0
Atrium	65.7	42.5	11.1	2.6	0.88

6 DAY OLD CHICK EMBRYOS

	1st day Av. Rate of beats per/min.	2nd day Av. Rate of beats per/min.	3rd day Av. Rate of beats per/min.	4th day Av. Rate of beats per/min.	5th day Av. Rate of beats per/min.
Sinus Venosus	70.1	58.1	7.5	0.15	0
Ventricle	51.6	33.9	7.5	0	0
Atrium	75.0	49.7	8.7	0	0